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A new species of *Nectria* on *Populus* from China

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Abstract – A new species, *Nectria zangii*, is described on *Populus* branches from Donglingshan in western Beijing. The anatomy of perithecia, which become cupulate when dry, and positive reactions to KOH and lactic acid of the fungus indicates that this species belongs in the genus *Nectria*. It is characterized by small, non-septate, allantoid ascospores and small, subcylindrical to narrowly clavate asci. Sequence analysis of the combined nuclear ribosomal DNA ITS1-5.8S-ITS2 and partial β-tubulin gene confirm its taxonomic position in *Nectria* as a species new to science.

Key words – taxonomy, morphology

Introduction

The genus *Nectria* (Fr.) Fr. was established in 1849 and typified by *N. cinnabarina* (Clements & Shear 1931). About 34 species are currently accepted in the genus (Rossman et al. 1999, Kirk et al. 2001, Döbbeler 2005a,b, Marincowitz et al. 2008, Pande 2008). Ten species are known from China (Teng 1963, Tai 1979, Wang et al. 1999, Zhuang & Zhang 2002, Zhang & Zhuang 2003a,b). The genus is characterized by well-developed stromata, subglobose, globose to ellipsoid, red to dark red, fleshy, soft-textured, uniloculate, warted perithecia that become cupulate when dry, and associated with coelomycetous anamorphs (Rossman et al. 1999, Hirooka et al. 2009). Ascospores of *Nectria* are variable and usually broadly ellipsoid to long-fusiform, hyaline to yellow-brown, smooth to striate, non- to multi-septate or muriform. Members of the genus are typically weak parasites of woody plants, and occur on hardwood trees and shrubs throughout temperate regions of the Northern Hemisphere (Samuels et al. 2009, Hirooka et al. 2011).

In this study, a new species of *Nectria* with small allantoid non-septate ascospores and subcylindrical narrow asci is described. On the basis of morphology and sequence analysis of nuclear ribosomal DNA ITS1-5.8S-ITS2

(ITS) and partial β-tubulin gene, its position in *Nectria* is confirmed, and its relationship with similar species of the genus is discussed.

Materials & methods

The taxonomic treatments and methods described in Luo & Zhuang (2010) were generally followed for morphological studies. Water was used as the mounting medium for microscopic examinations and measurements. Photographs were taken from water or lactophenol cotton blue mounts with a Canon G5 (Tokyo, Japan) digital camera connected to a Zeiss Axioskop 2 plus microscope (Göttingen, Germany). For anatomic studies, longitudinal sections through ascomata were made with a freezing microtome (YD-1508-III, Yidi Medical Appliance Factory, Jinhua, Zhejiang) at a thickness of ca. 8 µm. Continuous measurements of individual structures are based on 30 measurements, except when otherwise noted. Single-spore isolates were obtained from recent collections. Colony characters were recorded from cultures on potato dextrose agar (PDA, Gams et al. 1998). Microscopic descriptions of the anamorphs were taken from cultures after 14 days. Specimens examined are deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS), and cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences.

DNA was extracted from mycelium harvested from colonies on PDA after 2 weeks (Wang & Zhuang 2004). Sequences of ITS and the partial β-tubulin gene were amplified with primer pairs ITS5-ITS4 (White et al. 1990) and T1-T222 (O'Donnell & Cigelnik 1997) respectively. The PCR reaction mixture consisted of 2.5 µl 10× PCR buffer, 1.5 µl $MgCl_2$ (25 mM), 1.25 µl each primer (10 µM), 0.5 µl dNTP (10 mM each), 1.25 µl DNA

Table 1. Materials used in this study.

Species	Collection no.	ITS nrDNA GenBank no.	β-tubulin GenBank no.
Cosmospora coccinea Rabenh.	CBS 114050	HM484537	HM484589
Nectria australiensis Seifert	HMAS 83397	GU075855	HM054111
N. berolinensis (Sacc.) Cooke	CBS 126112	HM484543	HM484594
N. cinnabarina (Tode) Fr.	CBS 125150	HM484684	HM484820
	CBS 125158	HM484696	HM484830
N. coryli Fuckel	CBS 129156	HM484539	HM484596
N. miltina (Mont.) Mont.	CBS 121121	HM484547	HM484609
N. nigrescens Cooke	CBS 125148	HM484707	HM484806
	CBS 129360	HM484711	HM484808
N. pseudocinnabarina Rossman	CBS 128673	HM484553	HM484608
N. pseudotrichia (Schwein.) Berk. & M.A. Curtis	HMAS 183559	HM054154	HM054115
	HMAS 183175	HM054138	HM054114
N. zangii	HMAS 251258	**JN997424**[a]	**JN997421**
	holotype, HMAS 251247	**JN997425**	**JN997422**
	HMAS 188502	**JN997426**	**JN997423**
Neonectria ramulariae Wollenw.	CBS 151.29	HM054150	HM054124

[a] Numbers in bold = newly submitted sequences.

template, 0.25 µl Taq polymerase (5 U/µl) and 16.5 µl ddH_2O. Reactions were performed on an ABI 2720 Thermal Cycler (Gene Co. Ltd.) with cycling conditions of denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C (ITS region) and at 55°C (β-tubulin gene) for 30 s and elongation at 72°C for 45 s, with a final extension step at 72°C for 10 min to complete the reactions. PCR products were purified with the PCR Product Purification Kit (Biocolor BioScience & Technology Co.) and sequenced with the ABI BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730 XL DNA Sequencer (SinoGenoMax Co. Ltd.). ITS5 and ITS4 (for ITS), and T1 and Bt2b (for the partial β-tubulin gene) were employed as sequencing primers (White et al. 1990, Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). Sequences of related species were retrieved from GenBank and are listed in Table 1.

All sequences were aligned using ClustalX 1.8 (Thompson et al. 1997), and the alignments were visually adjusted by BioEdit 7.0.5 (Hall 1999) when necessary. A partition homogeneity test was performed with 1000 replicates in PAUP 4.0b10 (Swofford 2002) to evaluate statistical congruence between sequence data from ITS and β-tubulin gene regions. The partition homogeneity test (P = 0.01) suggested that the individual partitions were congruent (Farris et al. 1995, Cunningham 1997). A neighbor-joining tree was generated using MEGA 4.10 (Tamura et al. 2007) based on combined sequences of ITS and partial β-tubulin gene with *Cosmospora coccinea* and *Neonectria ramulariae* as the outgroup taxa. The Kimura 2-parameter was selected as the nucleotide substitution model, and gaps or missing data were pairwise deleted. Bootstrap analyses were performed with 1000 replicates to test phylogeny branch support.

Taxonomy

Nectria zangii Z.Q. Zeng & W.Y. Zhuang, **sp. nov.** Figure 1

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Differs from *Nectria miltina* by thinner perithecial walls, narrower asci, smaller ascospores, and occurrence on twigs of *Populus* sp.

Type: China. Beijing, Donglingshan, alt. 1150 m, on branches of *Populus* sp., 20 July 2011, Z.Q. Zeng & H.D. Zheng 7684 (**Holotype**, HMAS 251247).

Etymology: The specific epithet honors the late Chinese mycologist, Prof. M. Zang.

Ascomata perithecial, gregarious, up to 40 in a group, with a well-developed stroma that is erumpent through bark, superficial, subglobose to globose, 168–200 µm high, 151–203 µm diam., becoming cupulate upon drying, red when fresh and reddish brown when dry, turning dark red in 3% KOH aqueous solution and orange-red to orange in lactic acid, surface somewhat roughened when dry; with warts or a irregular covering layer of various thickness, 0–32 µm high, cells angular, 3.5–15 × 2.5–5 µm, wall 1–1.5 µm thick. Ascomatal wall 16–26 µm thick, of two layers; outer layer 10–16.5 µm thick, cells angular, 2–7 × 3–6 µm, wall 1–1.5 µm thick; inner layer 5–10.5 µm thick, cells flattened, 9.5–15.5 × 1.5–3.5 µm, wall 1–1.5 µm thick. Asci subcylindrical to narrowly clavate, 8-spored, with an apical ring, 28–35 × 2.5–3 µm (n = 50), biseriate

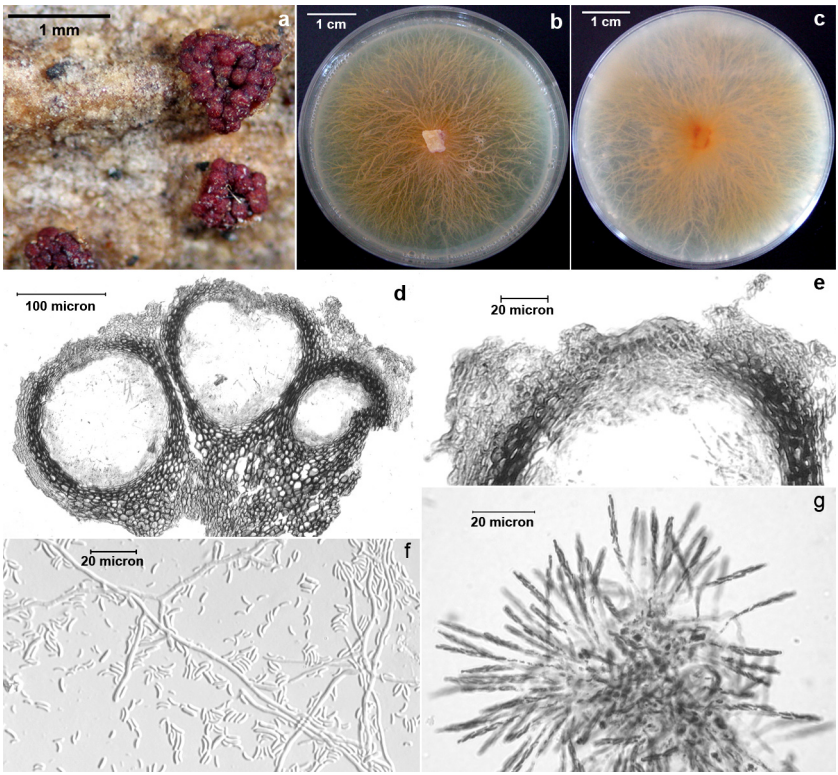

Fig. 1. *Nectria zangii* (holotype, HMAS 251247). a. Ascomata on the natural substrate. b. Colony on PDA, obverse. c. Colony on PDA, reverse. d. Median section of ascomata. e. Structure of ascoma at apical portion. f. Asci with ascospores. g. Conidiophores and conidia.

to irregularly biseriate. Ascospores allantoid to rod-shaped, straight to slightly curved, non-septate, hyaline, smooth, 3.5–5.5(–6) × 0.9–1.2(–1.4) μm (n = 50).

In culture, colonies reaching 57 mm in diameter after 7 days on PDA at 25°C under daylight, aerial mycelium sparse to absent, yellowish, submerged mycelia forming a root-like or vein-like structure, reverse light orange. Conidiophores unbranched to sparsely branched, septate, 29–79 × 2–3.2 μm, arising from agar surface throughout the colony, dominating near the margin. Microconidia allantoid to rod-shaped, not or slightly curved, hyaline, aseptate, 3–6 × 0.8–1 μm (n = 50). Macroconidia and chlamydospores not observed.

Additional specimens examined: **China**. **Beijing**, Donglingshan, alt. 1150m, on rotten branch of *Populus* sp., 20 July 2011, Z.Q. Zeng & H.D. Zheng 7648–7651 (HMAS 188501–188504); 7652 (HMAS 251248); 7653–7654 (HMAS 188505–188506); 7655 (HMAS 251249); 7656 (HMAS 188507); 7657–7660 (HMAS 251250–251253);

7663 (HMAS 188508); 7665–7667 (HMAS 188509–188511); 7668 (HMAS 251254); 7669–7671 (HMAS 188512–188514); 7672 (HMAS 251255); 7673–7680 (HMAS 188515–188522); 7681 (HMAS 251256); 7682 (HMAS 188523); 7683 (HMAS 251257); 7685–7694 (HMAS 188524–188533); 7695 (HMAS 251258); 7696–7698 (HMAS 188534–188536).

Comments – Morphologically, the perithecial anatomy, perithecia becoming cupulate when dry, and positive reaction to KOH and to lactic acid indicates the new species' position in *Nectria*. Among the existing species of the genus, *N. zangii* is most similar to *N. miltina* in shape of asci and ascospores. *Nectria miltina*, however, differs in having a thicker perithecial wall (25–35 μm thick), wider asci (28–36 × 3.5–5 μm), larger ascospores (5.5–7 × 1.5–2 μm), and an entirely different host. *Nectria miltina* occurs on leaves of the monocotyledonous plant family *Agavaceae* (Rossman et al. 1999) instead of on dicotyledonous *Populus* sp.

Sequence analysis of the combined ITS and partial β-tubulin gene from *N. zangii* and eight other *Nectria* species with various ascospore shapes and septation confirms taxonomic position of the new species (Fig. 2). Our results support two major distantly related clades within the sampled *Nectria* species. *Nectria zangii*, *N. berolinensis*, *N. miltina*, *N. coryli*, and *N. pseudocinnabarina* group together with high bootstrap support (99%). The latter four species were reported having sporodochial anamorphs (Rossman et al. 1999). The other

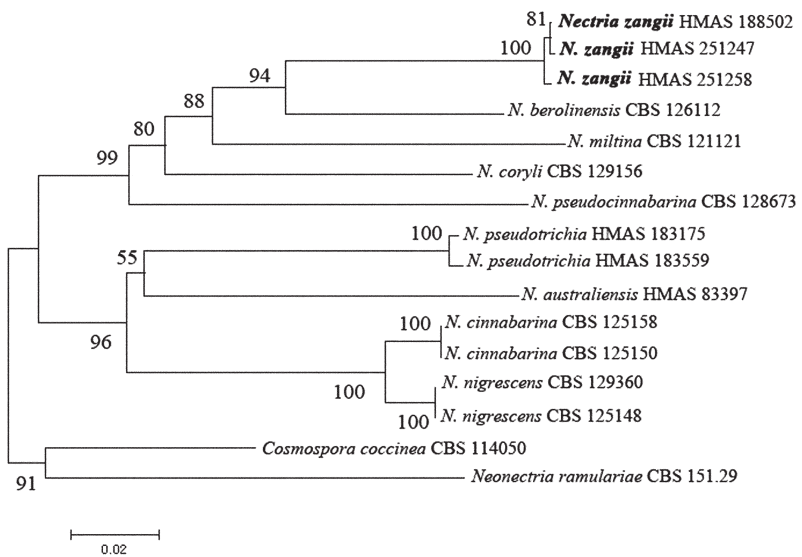

Fig. 2. Neighbor-joining tree based on combined sequences of ITS and partial β-tubulin gene showing the relationships among some *Nectria* species.

sampled species, including the type species *N. cinnabarina*, group together with 96% bootstrap support (Fig. 2). These results are identical to those by Hirooka et al. (2011). *Nectria berolinensis*, shown as sister to *N. zangii* with 94% bootstrap support, differs in larger perithecia (250–300 µm diam.) and 5–9-septate larger (16–20 × 7–8 µm) muriform elliptical ascospores (Seaver 1909). *Nectria miltina* also groups with *N. berolinensis* and *N. zangii* with relatively high support (88%). Thus, both morphology and DNA sequence analysis support the recognition of *N. zangii* as a new species.

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